IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re Application of:	Sándor Sipka et al.
Serial No:	10/651,136
Filed:	August 28, 2003
For:	Process for Inhibiting Allergic Disease
Art Unit:	1644
Examiner:	Nora M. Rooney

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

The present Appeal Brief is submitted in support of the Notice of Appeal filed by electronic filing on August 25, 2009.

I. REAL PARTIES IN INTEREST

The real parties in interest are the co-owners and inventors of the present application, Dr. Geza Bruckner and Dr. Sándor Sipka.

II. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences known to the Appellants or the Appellants' undersigned legal representative which will directly effect or be directly effected by or have a bearing on the Board's decision in the present appeal.

III. STATUS OF THE CLAIMS

Claims 1-3 and 5-18 and 20-25 are currently pending. Claims 6-9, 11-12, 14-16, and 20-21 stand withdrawn from further consideration as being drawn to nonelected species. Claims 1-3, 5, 10, 13, 17-18, and 22-25 are subject to examination in the present application. All pending claims subject to examination (1-3, 5, 10, 13, 17-18, and 22-25) stand rejected and are the subject of the present appeal. A complete copy of the pending claims is set forth in the Claims Appendix.

IV. STATUS OF AMENDMENT FILED SUBSEQUENT TO REJECTION ON APPEAL

Appellants have appealed the Examiner's final rejection of claims 1-3, 5, 10, 13, 17-18, and 22-25 set forth in the Official Action dated May 21, 2009 ("5-21 OA"). The last Amendment filed in this application was filed on March 2, 2009 and was acknowledged (and presumably entered) in the 5-21 OA.

V. SUMMARY OF CLAIMED SUBJECT MATTER

There are three independent claims. The embodiment of the invention defined by independent claim 1 provides a method for decreasing development of allergic asthma **(page 2, line 18 through page 3, line 2)**, the process comprising exposing a neonatal or immature mammal **(page 2, line 5, e.g.)** to irradiation-detoxified lipopolysaccharide (IR-LPS) **(page 2, lines 5-6, e.g.)** derived from extracted bacterial endotoxin **(page 4, lines 15-16, e.g.)** and operable to stimulate the Th 1 arm of the mammal's immune system **(page 4, lines 17-18)**, wherein exposure comprises at least weekly administration **(page 7, line 12)** during maturation

of the mammal **(page 7, line 10, e.g.)** via application of the IR-LPS to a living environment of the mammal **(page 6, line 24 through page 7, line 4, e.g.)**.

The embodiment of the invention defined by independent claim 22 provides a process for decreasing development of allergic asthma **(page 2, line 18 through page 3, line 2, e.g.)** in a mammal maturing in an overly sterile environment **(page 2, lines 9-11, e.g.)** by restoring normal immune system development **(page 2, lines 11-12)**, the process comprising exposing a neonatal or immature mammal **(page 2, line 19)** to irradiation-detoxified lipopolysaccharide **(page 2, line 20, e.g.)** derived from extracted E. coli bacteria endotoxin **(page 5, lines 4-8)** and operable to stimulate the Th 1 art of the mammal's immune system **(page 4, lines 17-18)**, wherein exposure occurs via administration of the IR-LPS during maturation of the mammal **(page 7, line 10, e.g.)**.

The embodiment of the invention defined by independent claim 25 provides a process for decreasing development of allergic asthma **(page 2, line 18 through page 3, line 2, e.g.)**, the process comprising exposing a neonatal or immature human **(page 2, line 19)** of up to about 2 years of age **(page 5, lines 22-23)** to irradiation-detoxified lipopolysaccharide **(page 2, line 20, e.g.)** derived from extracted bacterial endotoxin **(page 5, lines 4-8)** and operable to stimulate the Th 1 arm of the human's immune system **(page 4, lines 17-18)** while reducing interleukin 1 (IL-1) stimulation caused by the native form of the lipopolysaccharide derived from extracted bacterial endotoxin **(page 4, lines 1-3)**, wherein exposure comprises administration on an at least weekly basis **(page 7, lines 10-12)** of an aerosol spray composition **(page 6, line 24 through page 7, line 4)** comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 μg/ml **(page 8, Examples 2-4, e.g.).**

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

There are two issues on appeal for review by the Board, as follows:

A. The final rejection of claims 1-3, 5, 10, 13, 17-18, and 22-25 under 35 U.S.C. §103(a) as being unpatentable over Cochran et al., *Influence of Lipopolysaccharide Exposure on Airway Function and Allergic Responses in Developing Mice*, Pediatric Pulmonology 34: 267-77 (2002) (hereafter "Cochran") in view of Previte et al., *Detoxification on Salmonella typhimurium Lipopolysaccharide by Ionizing Radiation*, Journal of Bacteriology 93 (5): 1607-14 (1967) (hereafter "Previte").

B. The final rejection of claims 1-3, 5, 10, 13, 17-18, and 22-25 under 35 U.S.C. §103(a) as being unpatentable over Khan et al., *Functional and Immune Response to Lipopolysaccharide and Allergens in Developing Mice*, Pediatric Academic Societies' Annual Meeting Program Issue 51 (4) (2002) (hereafter "Khan") in view of Previte.

VII. GROUPING OF THE CLAIMS

A. With respect to the above noted issue **A** on appeal, Appellants concede that claims 1-3, 5, 10, 13, 17-18, and 22-25 stand or fall together.

B. With respect to the above noted issue **B** on appeal, Appellants concede that claims 1-3, 5, 10, 13, 17-18, and 22-25 stand or fall together.

VIII. ARGUMENT

Appellants' invention provides a process for decreasing development of allergic asthma by exposing a neonatal or immature mammal to irradiation-detoxified lipopolysaccharide (IR-LPS) derived from extracted bacterial endotoxin operable to stimulate the Th 1 arm of the

mammal's immune system. Exposure to the IR-LPS is accomplished through at least weekly administration during the maturation of the mammal via application of the IR-LPS to a living environment of the mammal. The invention provides an efficient method to decrease development of allergic asthma by treating the environment of a maturing mammal with IR-LPS throughout the maturing cycle of the mammal.

Cochran carried out experiments comprising administration of lipopolysaccharide (LPS) to 2-3 week old mice via a single intranasal instillation of LPS in saline. Cochran fails to teach administration of IR-LPS, administration of IR-LPS to the living environment of the mammal, or any method of administration other than a single, one-time dose directly to the nasal passage of the mammal. Cochran neither teaches nor suggests application of IR-LPS (or LPS) on an at least weekly basis or throughout the maturation of the mammal in order to decrease development of allergic asthma.

The Examiner applies Cochran for disclosing all the elements of instant claim 1 except the administration of IR-LPS and applies Previte, the secondary reference, for disclosure of IR-LPS, asserting that a person of ordinary skill in the art would be motivated to use the IR-LPS of Previte in the "method" of Cochran to decrease the known toxicity of native LPS.

Khan reported experiments comprising treating 3-week old mice with LPS intratracheally and measuring airway function. Like Cochran, Khan fails to teach administration of IR-LPS, administration of IR-LPS to the living environment of the mammal, or any method of administration other than a single, one-time dose directly to the nasal passage of the mammal. Khan neither teaches nor suggests application of IR-LPS (or LPS) on an at least weekly basis or throughout the maturation of the mammal in order to decrease development of allergic asthma.

The Examiner applies Khan for disclosing all the elements of instant claim 1 except the administration of IR-LPS and applies Previte, the secondary reference, for disclosure of IR-LPS, asserting that a person of ordinary skill in the art would be motivated to use the IR-LPS of Previte in the "method" of Khan to decrease the known toxicity of native LPS.

As set forth in detail below, the Examiner first fails to establish a *prima facie* case for obviousness under 35 U.S.C. § 103, since the combinations of Cochran or Khan and Previte fail to teach or suggest each element of Appellants' claimed invention. Further, Appellants assert that motivation to combine the references is absent, since Previte is directed to treatment of adult subjects only and teaches retention of an unacceptable degree of toxicity resulting in the death of 3/10 adult subjects 6 days post administration. Given the toxicity levels of Previte's IR-LPS, the ordinary skilled artisan would not be motivated to administer the toxic LPS of Previte to immature mammals, using the "methods" of Cochran or Khan. Moreover, the combinations of Cochran or Khan and Previte do not enable Appellant's claims, in that the mere insertion of Previte's IR-LPS into the "methods" of Cochran or Khan still does not yield a method requiring more than a single administration of LPS/IR-LPS to a maturing mammal, and does not teach administration to the living environment of the mammal. Finally, Appellants submit any assertion of obviousness is defeated by Appellants' secondary evidence of nonobviousness, showing the unexpectedly superior results of IR-LPS vs. LPS to protect against allergic disease. Reconsideration and reversal by the Board on the basis of the following arguments is respectfully requested.

A. The methods defined by claims 1-3, 5, 10, 13, 17-18, and 22-25 are nonobvious under 35 U.S.C. § 103 over Cochran in view of Previte

1. The Invention

The invention provides processes for decreasing development of allergic asthma in neonatal or immature mammals. The methods teach a non-invasive, user-friendly process for decreasing development of allergic asthma in immature mammals, including humans. The instant methods eliminate the need to directly dose a mammal, for example through injections or solutions instilled directly into the mammal's nasal passages. Rather, using the present inventive methods, one decreases the development of allergic asthma through repeated misting of the living environment of the mammal with a solution containing IR-LPS, which acts to prime the Th 1 arm of the mammal's immune system when passively inhaled.

The embodiment of the invention defined by independent claim 1 provides a process for decreasing allergic asthma in neonatal or immature mammals, comprising exposing a neonatal or immature mammal to irradiation-detoxified IR-LPS derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein the exposure comprises at least weekly administration of IR-LPS to the living environment of the mammal, during maturation of the mammal.

The embodiment of the invention defined by independent claim 22 provides a process for decreasing development of allergic asthma in a mammal maturing in an overly sterile environment by restoring normal immune system development, the process comprising exposing a neonatal or immature mammal to irradiation-detoxified lipopolysaccharide derived from extracted *E. coli* bacteria endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein exposure occurs via administration of the IR-LPS during maturation of the mammal.

The embodiment of the invention defined by independent claim 25 provides a process for decreasing development of allergic asthma, the process comprising exposing a neonatal or immature human of up to about 2 years of age to irradiation-detoxified lipopolysaccharide derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the human's immune system while reducing interleukin 1(IL-1) stimulation caused by the native form of the lipopolysaccharide derived from extracted bacterial endotoxin, wherein exposure comprises administration on an at least weekly basis of an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 μg/ml.

Surprisingly, the present inventors discovered that IR-LPS provides certain advantages over the use of native LPS, namely, that the prolonged pretreatment of the environment of infant mice with IR-LPS acts to prevent the intensity of allergic reaction differentially, as compared with native LPS. When administered on an at least weekly basis to the living environment of an immature mammal during the maturation period, the passive inhalation of the IR-LPS acts to stimulate the Th 1 arm of the mammal, thus decreasing development of allergic asthma safely and without the need for invasive treatment of the mammal itself.

2. The Examiner's Position

The Examiner asserts that Cochran teaches a process for decreasing development of allergic asthma (OVA induced asthma) comprising exposing an infant, neonatal or immature mammal maturing in an over sterile environment shortly after birth (2-3 week old laboratory mice) to lipopolysaccharide derived from extracted bacterial endotoxin (E. coli LPS) by administering an aerosol spray composition to a living environment/space of the mammal (saline and air during nasal aspiration) during maturation of the mammal (at 2-3 weeks), and also teaches that "recent studies raised the intriguing hypothesis that exposure to LPS may interact

with the immune system in early life and produce a protective environment against the development of asthma and atopy."

The Examiner notes that Cochran fails to teach any benefit or differential benefit or "irradiation detoxified lipopolysaccharide" as recited in claims 1-3, 5, 10, 17-18, and 22-25 "wherein exposure comprises at least weekly administration during maturation of the mammal" of claim 1; "wherein the irradiation-detoxified lipopolysaccharide is detoxified by exposure of the endotoxin to irradiation at a level of from about 25 to about 150 kGy" in claim 2; "wherein the irradiation changes the structure of the endotoxin while maintaining its Th1 stimulatory positive immune effect in the resulting irradiation-detoxified lipopolysaccharide" in claim 3; "wherein the mammal is a human and during maturation is between 1 month and 2 years of age" of claim 13; "during maturation is throughout the maturing life cycle of the mammal" in claim 17; "wherein administration is on a daily basis" of claim 18; "wherein the mammal is a human infant and exposure comprises at least weekly administration from about 1 month to 2 years of age" of claim 24; "and exposing a "human of up to about 2 years of age" and "wherein exposure comprises administration on an at least weekly basis of an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 μg/ml" in claim 25.

The Examiner applies Previte for teaching the detoxification of isolated LPS of *S. typhimurium*, *S. enteritidis* and *E. coli* using 4, 4.8 and 4.5 Mrad (about 25 to about 150 kGy) ionizing radiation to ostensibly eliminate lethality induced by its lethal determinants (which the Examiner asserts to be a result of changes in the structure), while retaining antigenicity (which the Examiner asserts to be via maintaining its Th1 stimulatory effects) and pyrogenicity. It is significant to note that Previte predates Cochran by 35 years.

The Examiner asserts that no patentable weight may be assessed with respect to the functional limitation described by the recitation of "operable to stimulate the Th1 arm of the human's immune system" of claims 1 and 22; and "operable to stimulate the Th1 arm of the human's immune system while reducing interleukin 1 (IL-1) stimulation caused y the native form of the lipopolysaccharide derived from extracted bacterial endotoxin" of claim 25; and "by restoring normal immune system development" in claim 22. The Examiner further asserts that these are inherent properties of the referenced irradiation-detoxified lipopolysaccharide. The Examiner include claims 1-3, 5, 10, 17-18, and 22-25 in the rejection as requiring no more than identification and determination of optimum modes and workable ranges involving only routine skill in the art.

The Examiner asserts that it would be obvious to practice the process taught by Cochran in humans of 1 month to 2 years of age and during the maturing life cycle of the mammal because Cochran suggests performing the process for decreasing development of allergic asthma in children under 5 years of age implicitly. The Examiner further asserts that a person of ordinary skill would be motivated to use the irradiation detoxified LPS of Previte in processes for decreasing allergic asthma of Cochran "because the process should be safe and without toxic effects for use in infants and children." Previte teaches that LPS can be irradiation detoxified for its lethal determinants "while still retaining antigenicity and pyrogenicity." The Examiner argues that it would be obvious to use a safer, less toxic form of LPS in neonate or immature mammals.

3. Appellants' Arguments

a. <u>The Examiner fails to establish a *prima facie* case for obviousness under 35 U.S.C. § 103 because all claim limitations are not taught or suggested by the</u>

combination of Cochran and Previte and motivation to combine the references is absent.

To establish *prima facie* obviousness of the claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Summarily, Appellants submit that Cochran fails to teach or suggest at least three essential steps of the instant invention. First, as noted by the Examiner, Cochran used LPS rather than IR-LPS in his experiments. Second, Cochran teaches only a one-time exposure to an immature mammal, while Appellants teach repeated exposure over a critical maturation period. Third, Cochran teaches application of LPS directly to the subject (via intranasal instillation), while Appellants teach indirect exposure by application of IR-LPS to the living environment of the mammal. All of these elements are disclosed by Appellants as critical to the efficacy of the inventive method, yet the Examiner dismisses the absence of the latter two, and applies Previte - a reference that teaches only direct administration of IR-LPS to adult mammals to test for a decrease in toxicity, reporting death of nearly a third of the subjects 6 days post-administration (see, e.g., Fig. 3), and "extensive inactivation of antigenic components with increasing radiation dose" (page 1611, second column, line 11-14).

Appellants note that if it were true that the disclosure of Previte would guide an ordinary practitioner to the use of IR-LPS in the methods of Cochran (as the Examiner asserts), then surely Cochran himself would have employed IR-LPS since Previte's findings were published some 35 years prior to Cochran. However, even if the combination of references were proper, which Appellants contend it is not, Previte simply does not overcome the deficiencies of Cochran. At least two elements of the instant invention remain wholly unaddressed by the combined references: the passive administration of IR-LPS to the living environment of the

mammal, and the repeated administration of IR-LPS over a period of maturation of the mammal ("at least weekly" in independent claims 1 and 25 and "during maturation of the mammal" in independent claim 22).

All claim limitations must be considered in an obviousness rejection. 35 U.S.C. § 103 provides that:

> A patent may not be obtained . . . if the differences between the subject matter sought to be patented and the prior art are such that the subject matter **as a whole** would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

(Emphasis added). Since Cochran and Previte together completely fail to address administration of IR-LPS to the environment of the mammal, through repeated doses during the maturation period of the mammal, Appellants assert the combined references fail to render obvious the subject matter as a whole. Absent any teaching or suggestion of the missing claim elements, Appellants submit the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. § 103.

Moreover, there must be a teaching or suggestion within the prior art, within the nature of the problem to be solved, or within the general knowledge of a person of ordinary skill in the field of the invention, to look to particular sources, to select particular elements, and to combine them as combined by the inventor. *See Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 665, 57 USPQ2d 1161, 1167 (Fed. Cir. 2000). Appellants note that not only does Previte fail to disclose or suggest the missing elements of administration across a maturation period and indirect exposure to the environment as the form of administration, Previte in fact discloses the retention of a degree of lethality upon direct administration to adult mammals that would certainly guide a practitioner away from direct administration to an immature subject. Indeed, Previte reports a death rate of 3/10 adult subjects 6 days post-administration. And yet, the Examiner expressly

states the motivation for using Previte's IR-LPS in the method of Cochran is specifically because a practitioner would conclude, based on the teachings of Previte, that the method would be "safe" for children and infants. The Examiner's assertion that the motivation to combine the references is "because it would be safe for children" is untenable, given the reported death rate associated with Previte's findings. Appellants contend that a positive death rate of 3/10 adult subjects predictably due to the treatment, as disclosed by Previte for IR-LPS levels within the scope of the instant invention, would be universally understood as unacceptable. Rather, a person of ordinary skill in the art seeking methods to prophylactically decrease development of allergic asthma would be discouraged from employing the IR-LPS of Previte into the protocol of Cochran, as Previte teaches a single relative high dose to adult rats which results in an unacceptably high death rate among the subjects.

The Examiner contends that, because IR-LPS is the active, therefore all effects on the subjects are inherent. In taking this position, though, the Examiner fails to consider the distinguishing impact of the route and manner of administration. The Examiner cannot fairly conclude that the two methods are identical with respect to toxicity of the active, antigenicity of the active, or with respect to achieving the target outcome. Indeed, Appellants note that Previte supports this proposition, since Previte discloses a single dose of IR-LPS administered directly to the subject, which results in toxicity to a relatively high percentage of subjects, whereas the instant inventive methods rely on repeated passive dosing during the maturation period of the mammal by misting the environment without toxic effect. Clearly, given these disparate results, route and manner of administration are distinguishing factors that lead to different toxicity and antigenicity outcomes.

In summary, Appellants assert the combination of Cochran and Previte fails to teach or suggest at least two important claim limitations -- administration of IR-LPS repeatedly, during the maturation of the mammal, and indirect administration via treatment of the living environment of the mammal. Moreover, motivation to combine the references is absent, given Previte's unacceptably high reported death rate of 3/10 adult subjects. Appellants submit a *prima facie* case for obviousness under 35 U.S.C. § 103 has not been established by the Examiner.

b. Cochran and Previte do not enable the instant inventive methods, since the combination of references does not place the claimed invention in the possession of the public.

To render a later invention unpatentable for obviousness, the prior art must enable a person of ordinary skill in the art to make and use the later invention. The prior art must place the claimed invention in the possession of the public. *Beckman Instruments, Inc. v. LKB Produktor LB,* 892 F.2d 1547, 1551 (Fed. Cir. 1989). Appellants submit the combination of Cochran and Previte does not enable the instant invention, since all claim elements are not taught or suggested by the references and one of ordinary skill in the art could not derive the claimed methods from the cited references without undue experimentation.

Even if the combination of Cochran and Previte were proper (which Appellants contend it is not, in detail above), the combination itself still fails to enable the instant inventive methods. Combining the IR-LPS of Previte with the protocol of Cochran still enables, at best, nothing more than a single dose of IR-LPS, administered invasively to a mammal via intra-nasal instillation of a solution comprising IR-LPS. Appellants note that the ultimate findings of Cochran teach only that a "single airway exposure to LPS in young mice leads to airway

hyperresponsiveness." (Cochran, p. 272, col. 1, lines 6-8). Appellants' methods, however, require administration of IR-LPS to the living environment of the mammal through repeated treatments during the maturation of the mammal.

Deriving the instant methods from a reading of Cochran and Previte requires altering both mode and frequency of administration, either one of which would require undue experimentation on the part of the ordinary skilled artisan to achieve. That is, a practitioner would need to first conceive of the idea of administering IR-LPS to the living environment of the mammal, rather than direct intra-nasal instillation (Cochran) or injection (Previte) of the mammal itself, without any guidance or direction whatsoever in the cited references. Then, the practitioner would need to experiment with different dosing regimens - varying from the single doses described in both Cochran and Previte - in order to determine the present inventive method of decreasing development of allergic asthma in the mammal. Given the unpredictability in the art, the absence of direction in Cochran and Previte, and the quantity of experimentation needed relative to the references, Appellants contend such a leap would necessarily require undue experimentation on the part of the ordinary skilled artisan.

Since Cochran and Previte together fail to place the present invention in the possession of the public, Appellants submit the instant inventive methods are not enabled by Cochran and Previte.

c. Secondary evidence of nonobviousness rebuts any *prima facie* case and demonstrates the unexpectedly superior results of IR-LPS relative to LPS in the methods of the instant invention.

Even if a *prima facie* case of obviousness under § 103 were established, Appellants' secondary evidence of nonobviousness rebuts the case. The Declaration of Dr. Sándor Sipka, M.D., Ph.D., executed February 23, 2009, filed March 2, 2009 ("The March 2009 Declaration"), included herewith in the Evidence Appendix, demonstrates unexpected results and must be afforded due consideration.

i) The evidence should be afforded <u>substantial weight</u> because a nexus exists between the claimed invention and the evidence of unexpected results provided in the March 2009 Declaration.

In the March 2009 Declaration, Dr. Sipka described experimental protocols and results relating to comparing the *in vivo* immunomodulatory effects of IR-LPS versus LPS when administered in accordance with the instant invention (as a mist sprayed into the environment). As stated by Dr. Sipka, the results clearly demonstrate that "prolonged pretreatment of the environment of infant mice with IR-LPS acts to prevent the intensity of ragweed specific allergic reaction differentially when compared to native LPS" (page 3, paragraph 6).

The Examiner has asserted that the Declaration does not provide evidence of an unpredicted differential impact of IR-LPS over LPS. The Examiner merely dismissed the data set forth in the Declaration as "neither surprising nor … commensurate in scope with the claims, which are directed to a method of decreasing development of allergic asthma in neonatal or immature mammals by administration to a living environment of the mammal at least weekly."

To be given substantial weight in the determination of obviousness or nonobviousness, evidence of secondary considerations must be relevant to the subject matter as claimed. The examiner must determine whether there is a nexus between the merits of the claimed invention

and the evidence of secondary considerations. *Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*, 776 F.2d 281, 305, 227 USPQ 657, 673-674 (Fed. Cir. 1985), *cert. denied*, 475 U.S. 1017 (1986). The term "nexus" designates a factually and legally sufficient connection between the objective evidence of nonobviousness and the claimed invention so that the evidence is of probative value in the determination of nonobviousness. *Demaco Corp. v. F. Von Langsdorff Licensing Ltd.*, 851 F.2d 1387, 7 U.S.P.Q.2d 1222 (Fed. Cir.), *cert. denied*, 488 U.S. 956 (1988).

Appellants submit the March 2009 Declaration is most certainly relevant to the instantly claimed methods. The experimental method reported in the March 2009 Declaration aligns with the present claims, including (1) exposing immature mammals (6 week mice at beginning of treatment) to either IR-LPS or LPS (2) on an at least weekly basis (daily for eight weeks, during the maturation period of the mammal) (3) to the living environment of the mammal (misting the cages). In order to assess the effects on development of allergic disease, animals were then sensitized with ragweed allergen and later challenged with the allergen. Macrophage and neutrophil counts were determined for bronchial lavage samples (BAL), as well as cytokine concentrations for TNF-α (a TH 1 cytokine), IL-4, and IL-5 (a Th 2 cytokine). (See March 2009 Declaration, page 2, paragraph 4). The effect on allergic disease development was evaluated by assessing these indicators of allergic disease -- macrophage and neutrophil numbers, as well as *in vivo* immunomodulatory effects on cytokines, particularly the TH 1 cytokine, TNF-α.

Clearly, a nexus exists between the claimed invention, which provides methods for decreasing allergic asthma, and the data, which demonstrate the comparative unexpected superiority of IR-LPS in protecting against allergic disease, as evidenced by analysis of macrophage and neutrophil numbers as well as TNF-α levels. That is, a legally and factually sufficient connection between the claimed invention and the objective evidence of

nonobviousness is present, such that the evidence should be considered in the determination of nonobviousness. Given the nexus between Dr. Sipka's data and the instant claimed invention, Appellants submit the Examiner erred in failing to consider and afford proper weight to the factual evidence provided by Dr. Sipka in the March 2009 Declaration.

ii) The surprisingly superior effect of IR-LPS over native LPS provides evidence of unexpected results that rebuts any *prima facie* case of obviousness.

Appellants submit the results reported in the March 2009 Declaration provide evidence of the surprising superiority of IR-LPS compared to native LPS, with respect to stimulating the TH 1 arm of the immune system and protecting against hyper-immune response to an allergen. According to Dr. Sipka, the results illustrate "a striking difference between the *in vivo* immunomodulatory effects of IR-LPS and native LPS on macrophage and neutrophil numbers," (March 2009 Declaration, page 3, paragraph 6). Further, TNF-α levels were increased significantly by 3.56 fold compared to controls for the IR-LPS, as compared with 1.66 fold for native LPS (see March 2009 Declaration, page 3, paragraph 6). Dr. Sipka specifically stated the results indicate a "surprisingly superior effect of IR-LPS over LPS in protecting against the development of hyper-immune response to an allergen neither taught nor suggested by any of the prior art" cited by the Examiner or known to him (March 2009 Declaration, page 4, paragraph 7).

Neither Cochran nor Previte teach or suggest the superior effects of IR-LPS compared to native LPS in protecting against the development of allergic disease. Accordingly, Appellants submit the March 2009 Declaration provided by Dr. Sipka constitutes secondary evidence of nonobviousness rebutting any *prima facie* case of obviousness, since it clearly demonstrates that,

specifically with respect to the methods of the instant invention, IR-LPS yields unexpectedly superior results in decreasing allergic response, relative to native LPS.

For the reasons set forth above, Appellants respectfully request that the Board reverse the final rejection of claims 1-3, 5, 10, 17-18, and 22-25 as being obvious under 35 U.S.C. § 103 over Cochran in view of Previte.

B. The methods defined by claims 1-3, 5, 10, 13, 17-18, and 22-25 are nonobvious under 35 U.S.C. §103 over Khan in view of Previte.

1. The Invention

The invention provides processes for decreasing development of allergic asthma in neonatal or immature mammals. The methods teach a non-invasive, user-friendly process for decreasing development of allergic asthma in immature mammals, including humans. The instant methods eliminate the need to directly dose a mammal, for example through injections or solutions instilled directly into the mammal's nasal passages. Rather, using the present inventive methods, one decreases the development of allergic asthma through repeated misting of the living environment of the mammal with a solution containing IR-LPS, which acts to prime the Th 1 arm of the mammal's immune system when passively inhaled.

The embodiment of the invention defined by independent claim 1 provides a process for decreasing allergic asthma in neonatal or immature mammals, comprising exposing a neonatal or immature mammal to irradiation-detoxified IR-LPS derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein the exposure

comprises at least weekly administration of IR-LPS to the living environment of the mammal, during maturation of the mammal.

The embodiment of the invention defined by independent claim 22 provides a process for decreasing development of allergic asthma in a mammal maturing in an overly sterile environment by restoring normal immune system development, the process comprising exposing a neonatal or immature mammal to irradiation-detoxified lipopolysaccharide derived from extracted *E. coli* bacteria endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein exposure occurs via administration of the IR-LPS during maturation of the mammal.

The embodiment of the invention defined by independent claim 25 provides a process for decreasing development of allergic asthma, the process comprising exposing a neonatal or immature human of up to about 2 years of age to irradiation-detoxified lipopolysaccharide derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the human's immune system while reducing interleukin 1(IL-1) stimulation caused by the native form of the lipopolysaccharide derived from extracted bacterial endotoxin, wherein exposure comprises administration on an at least weekly basis of an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 μg/ml.

Surprisingly, the present inventors discovered that IR-LPS provides certain advantages over the use of native LPS, namely, that the prolonged pretreatment of the environment of infant mice with IR-LPS acts to prevent the intensity of allergic reaction differentially, as compared with native LPS. When administered on an at least weekly basis to the living environment of an immature mammal during the maturation period, the passive inhalation of the IR-LPS acts to

stimulate the Th 1 arm of the mammal, thus decreasing development of allergic asthma safely and without the need for invasive treatment of the mammal itself.

2. The Examiner's Position

Claims 1-3, 5, 10, 13, 17-18, and 22-25 stand rejected under 35 U.S.C. § 103 as being obvious over Khan in view of Previte. The Examiner applies Khan for teaching "a process for decreasing development of allergic asthma (OVA induced asthma) comprising exposing an infant, neonatal or immature mammal maturing in an overly sterile environment shortly after birth (3 week old laboratory mice) to lipopolysaccharide derived from extracted bacterial endotoxin (LPS) by administering an aerosol spray composition to a living environment/space of a mammal (saline and air during intratracheal aspiration) during maturation of the mammal (at 3 weeks)." The Examiner states that Khan discloses that "recent evidence has suggested that post-natal exposure to endotoxin may protect against the development of allergen sensitization and asthma." The Examiner notes that Khan fails to teach IR-LPS, "at least weekly administration during maturation of the mammal," "a detoxification radiation level of from about 25 to 150 kGy," "wherein the irradiation changes the structure of the endotoxin while maintaining its Th1 stimulatory positive immune effect in the resulting irradiation-detoxified lipopolysaccharide," "wherein the mammal is a human and during maturation is between 1 month and 2 years of age," "wherein administration is on a daily basis," "wherein the mammal is a human infant and exposure comprises at least weekly administration from 1 month to 2 years of age," "exposing a human of up to about 2 years of age" and "wherein exposure comprises administration on at least weekly basis of an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 ug/ml" in claim 25.

The Examiner applies Previte for teaching the detoxification of isolated LPS of *S. typhimurium*, *S. enteritidis* and *E. coli* using 4, 4.8 and 4.5 Mrad (about 25 to about 150 kGy) ionizing radiation to ostensibly eliminate lethality induced by its lethal determinants (which the Examiner asserts to be a result of changes in the structure), while retaining antigenicity (which the Examiner asserts to be via maintaining its Th1 stimulatory effects) and pyrogenicity. With respect to these last ostensible teachings, the Examiner asserts that these are disclosed inherently even though dosing, routes of administration, and maturation status of the subjects are all significantly different. As was the case with Cochran, Appellants again note that Previte predates Khan by 35 years.

The Examiner argues that since Khan notes that "recent evidence has suggested that post-natal exposure to endotoxin may protect against the development of allergen sensitization and asthma," practice of the methods in humans of 1 month to 2 years of age and during maturation would be obvious. The Examiner argues that a person of ordinary skill in the art would have been motivated to use the IR-LPS of Previte in the Khan process for decreasing allergic asthma because the process "should be safe and without toxic effects for use in infants and children." The Examiner has stated that Khan "teaches that toxicity is decreased and that teaching alone provides motivation to use irradiated LPS in place of LPS" and further notes that "LPS is fully toxic and is being used medically in both the Previte et al. and Khan et al. references."

3. Appellants' Arguments

a. <u>The Examiner fails to establish a *prima facie* case for obviousness under 35 U.S.C. § 103 because all claim limitations are not taught or suggested by the combination of Khan and Previte and motivation to combine the references is absent.</u>

To establish *prima facie* obviousness of the claimed invention, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). Summarily, Appellants submit that Khan fails to teach or suggest at least three essential steps of the instant invention. First, as noted by the Examiner, Khan used LPS rather than IR-LPS in his experiments. Second, Khan teaches only a one-time exposure to an immature mammal, while Appellants teach repeated exposure over the maturation period. Third, Khan teaches application of LPS directly to the subject (via intratracheal administration), while Appellants teach indirect exposure by application of IR-LPS to the living environment of the mammal. All of these elements are disclosed by Appellants as critical to the efficacy of the inventive method, yet the Examiner again dismisses the absence of the latter two, and applies Previte - a reference that teaches only direct administration of IR-LPS to adult mammals to test for a decrease in toxicity, reporting death of nearly a third of the subjects 6 days post-administration (see, e.g., Fig. 3), and "extensive inactivation of antigenic components with increasing radiation dose" (page 1611, second column, line 11-14).

Even if the combination of references were proper, which Appellants contend it is not, Previte simply does not overcome the deficiencies of Khan. At least two elements of the instant invention remain wholly unaddressed by the combined references: the passive administration of IR-LPS to the living environment of the mammal, and the repeated administration of IR-LPS over a period of maturation of the mammal ("at least weekly" in independent claims 1 and 25 and "during maturation of the mammal" in independent claim 22).

All claim limitations must be considered in an obviousness rejection. 35 U.S.C. § 103 provides that:

> A patent may not be obtained . . . if the differences between the subject matter sought to be patented and the prior art are such that the subject matter **as a whole**

> would have been obvious at the time the invention was made to a person having ordinary skill in the art to which the subject matter pertains.

(Emphasis added). Since Khan and Previte together completely fail to address administration of IR-LPS to the environment of the mammal, through repeated doses during the maturation period of the mammal, Appellants assert the combined references fail to render obvious the subject matter as a whole. Absent any teaching or suggestion of the missing claim elements, Appellants submit the Examiner has failed to establish a *prima facie* case of obviousness under 35 U.S.C. § 103.

Further, a prior art reference must be considered in its entirety, i.e., as a whole, including portions that would lead away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). Appellants submit that Khan explicitly teaches away from the use of LPS in reducing subsequent allergic responses, and since Khan states that "airway exposure to LPS produces transient AHR (airway hyperresponsiveness) and inflammation in developing mice and does not appear to influence functional and immune responses induced by subsequent allergen sensitization" (Khan, last paragraph, emphasis added). The Examiner contends that Khan "is being relied upon for its specific teachings, namely the administration of LPS to neonatal or immature mammals to decrease development of allergic asthma." However, the Examiner's application of Khan completely disregards the ultimate conclusion of Khan, which is that LPS produces only transient airway hyperresponsiveness and does not appear to influence later functional and immune responses to allergic challenge. Indeed, Khan expressly teaches that LPS does not appear to influence the *very responses* sought to be elicited by the present invention. Accordingly, Appellants submit Khan in fact teaches away from the present inventive methods.

Moreover, there must be a teaching or suggestion within the prior art, within the nature of the problem to be solved, or within the general knowledge of a person of ordinary skill in the field of the invention, to look to particular sources, to select particular elements, and to combine them as combined by the inventor. *See Ruiz v. A.B. Chance Co.*, 234 F.3d 654, 665, 57 USPQ2d 1161, 1167 (Fed. Cir. 2000). Appellants note that not only does Previte fail to disclose or suggest the missing elements of administration across a maturation period and indirect exposure to the environment as the form of administration, Previte in fact discloses the retention of a degree of lethality upon direct administration to adult mammals that would certainly guide a practitioner away from direct administration to an immature subject. Indeed, Previte reports a death rate of 3/10 adult subjects 6 days post-administration. And yet, the Examiner expressly states the motivation for using Previte's IR-LPS in the method of Khan is specifically because a practitioner would conclude, based on the teachings of Previte, that the method would be "safe" for children and infants. The Examiner's assertion that the motivation to combine the references is "because it would be safe for children" is untenable, given the reported death rate associated with Previte's findings. Appellants contend that a positive death rate of 3/10 adult subjects predictably due to the treatment, as disclosed by Previte for IR-LPS levels within the scope of the instant invention, would be universally understood as unacceptable. Rather, a person of ordinary skill in the art seeking methods to prophylactically decrease development of allergic asthma would be discouraged from employing the IR-LPS of Previte into the protocol of Khan, as Previte teaches a single relative high dose to adult rats which results in an unacceptably high death rate among the subjects.

The Examiner contends that, because IR-LPS is the active, therefore all effects on the subjects are inherent. In taking this position, though, the Examiner fails to consider the

distinguishing impact of the route and manner of administration. The Examiner cannot fairly conclude that the two methods are identical with respect to toxicity of the active, antigenicity of the active, or with respect to achieving the target outcome. Indeed, Appellants note that Previte supports this proposition, since Previte discloses a single dose of IR-LPS administered directly to the subject, which results in toxicity to a relatively high percentage of subjects, whereas the instant inventive methods rely on repeated passive dosing during the maturation period of the mammal by misting the environment without toxic effect. Clearly, given these disparate results, route and manner of administration are distinguishing factors that lead to different toxicity and antigenicity outcomes.

In summary, Appellants assert the combination of Khan and Previte fails to teach or suggest at least two important claim limitations - administration of IR-LPS repeatedly, during the maturation of the mammal, and indirect administration via treatment of the living environment of the mammal. Moreover, Khan in fact teaches away from the present inventive methods, and motivation to combine the references is absent, given Previte's unacceptably high reported death rate of 3/10 adult subjects. Appellants submit a *prima facie* case for obviousness under 35 U.S.C. § 103 has not been established by the Examiner.

b. Khan and Previte do not enable the instant inventive methods, since the combination of references does not place the claimed invention in the possession of the public.

To render a later invention unpatentable for obviousness, the prior art must enable a person of ordinary skill in the art to make and use the later invention. The prior art must place the claimed invention in the possession of the public. *Beckman Instruments, Inc. v. LKB*

Produktor LB, 892 F.2d 1547, 1551 (Fed. Cir. 1989). Appellants submit the combination of Khan and Previte does not enable the instant invention, since all claim elements are not taught or suggested by the references and one of ordinary skill in the art could not derive the claimed methods from the cited references without undue experimentation.

Even if the combination of Khan and Previte were proper (which Appellants contend it is not, in detail above), the combination itself still fails to enable the instant inventive methods. Combining the IR-LPS of Previte with the protocol of Khan still enables, at best, nothing more than a single dose of IR-LPS, administered invasively to a mammal via intratracheal administration of a solution comprising IR-LPS. Appellants note that the ultimate findings of Khan teach only that a "airway exposure to LPS produces transient AHR (airway hyperresponsiveness) and inflammation in developing mice" and, importantly, "does not appear to influence functional and immune responses induced by subsequent allergen sensitization (Khan, last paragraph, emphasis added). Appellants' methods, however, require administration of IR-LPS to the living environment of the mammal through repeated treatments during the maturation of the mammal in order to decrease development of allergic asthma.

Deriving the instant methods from a reading of Khan and Previte requires altering both mode and frequency of administration, either one of which would require undue experimentation on the part of the ordinary skilled artisan to achieve, as well as conjuring an expectation of success in spite of Khan's teaching away from any such expectation. A practitioner would need to conceive of the idea of administering IR-LPS to the living environment of the mammal, rather than direct intratracheal instillation (Khan) or injection (Previte) of the mammal itself, without any guidance or direction whatsoever in the cited references. Then, the practitioner would need to experiment with different dosing regimens - varying from the single doses described in both

Khan and Previte - in order to determine the present inventive method of decreasing development of allergic asthma in the mammal. Given the unpredictability in the art, the absence of direction in Khan and Previte, Khan's express teaching away, and the quantity of experimentation needed relative to the references, Appellants contend such a leap would necessarily require undue experimentation on the part of the ordinary skilled artisan.

Since Khan and Previte together fail to place the present invention in the possession of the public, Appellants submit the instant inventive methods are not enabled by Khan and Previte.

c. Secondary evidence of nonobviousness demonstrates the unexpectedly superior results of IR-LPS relative to LPS in the methods of the instant invention.

Even if a *prima facie* case of obviousness under § 103 were established, Appellants' secondary evidence of nonobviousness rebuts the case. The Declaration of Dr. Sándor Sipka, M.D., Ph.D., executed February 23, 2009, filed March 2, 2009 ("The March 2009 Declaration"), included herewith in the Evidence Appendix, demonstrates unexpected results and must be afforded due consideration.

i) The evidence should be afforded substantial weight because a nexus exists between the claimed invention and the evidence of unexpected results provided in the March 2009 Declaration.

In the March 2009 Declaration, Dr. Sipka described experimental protocols and results relating to comparing the *in vivo* immunomodulatory effects of IR-LPS versus LPS when administered in accordance with the instant invention (as a mist sprayed into the environment).

As stated by Dr. Sipka, the results clearly demonstrate that "prolonged pretreatment of the environment of infant mice with IR-LPS acts to prevent the intensity of ragweed specific allergic reaction differentially when compared to native LPS" (page 3, paragraph 6).

The Examiner has asserted that the Declaration does not provide evidence of an unpredicted differential impact of IR-LPS over LPS. The Examiner merely dismissed the data set forth in the Declaration as "neither surprising nor … commensurate in scope with the claims, which are directed to a method of decreasing development of allergic asthma in neonatal or immature mammals by administration to a living environment of the mammal at least weekly."

To be given substantial weight in the determination of obviousness or nonobviousness, evidence of secondary considerations must be relevant to the subject matter as claimed. The examiner must determine whether there is a nexus between the merits of the claimed invention and the evidence of secondary considerations. *Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*, 776 F.2d 281, 305, 227 USPQ 657, 673-674 (Fed. Cir. 1985), *cert. denied*, 475 U.S. 1017 (1986). The term "nexus" designates a factually and legally sufficient connection between the objective evidence of nonobviousness and the claimed invention so that the evidence is of probative value in the determination of nonobviousness. *Demaco Corp. v. F. Von Langsdorff Licensing Ltd.*, 851 F.2d 1387, 7 U.S.P.Q.2d 1222 (Fed. Cir.), *cert. denied*, 488 U.S. 956 (1988).

Appellants submit the March 2009 Declaration is most certainly relevant to the instantly claimed methods. The experimental method reported in the March 2009 Declaration aligns with the present claims, including (1) exposing immature mammals (6 week mice at beginning of treatment) to either IR-LPS or LPS (2) on an at least weekly basis (daily for eight weeks, during the maturation period of the mammal) (3) to the living environment of the mammal (misting the

cages). In order to assess the effects on development of allergic disease, animals were then sensitized with ragweed allergen and later challenged with the allergen. Macrophage and neutrophil counts were determined for bronchial lavage samples (BAL), as well as cytokine concentrations for TNF-α (a TH 1 cytokine), IL-4, and IL-5 (a Th 2 cytokine). (See March 2009 Declaration, page 2, paragraph 4). The effect on allergic disease development was evaluated by assessing these indicators of allergic disease -- macrophage and neutrophil numbers, as well as *in vivo* immunomodulatory effects on cytokines, particularly the TH 1 cytokine, TNF-α.

Clearly, a nexus exists between the claimed invention, which provides methods for decreasing allergic asthma, and the data, which demonstrate the comparative unexpected superiority of IR-LPS in protecting against allergic disease, as evidenced by analysis of macrophage and neutrophil numbers as well as TNF-α levels. That is, a legally and factually sufficient connection between the claimed invention and the objective evidence of nonobviousness is present, such that the evidence should be considered in the determination of nonobviousness. Given the nexus between Dr. Sipka's data and the instant claimed invention, Appellants submit the Examiner erred in failing to consider and afford proper weight to the factual evidence provided by Dr. Sipka in the March 2009 Declaration.

ii) The surprisingly superior effect of IR-LPS over native LPS provides evidence of unexpected results that rebuts any *prima facie* case of obviousness.

Appellants submit the results reported in the March 2009 Declaration provide evidence of the surprising superiority of IR-LPS compared to native LPS, with respect to stimulating the TH 1 arm of the immune system and protecting against hyper-immune response to an allergen.

According to Dr. Sipka, the results illustrate "a striking difference between the *in vivo* immunomodulatory effects of IR-LPS and native LPS on macrophage and neutrophil numbers," (March 2009 Declaration, page 3, paragraph 6). Further, TNF-α levels were increased significantly by 3.56 fold compared to controls for the IR-LPS, as compared with 1.66 fold for native LPS (see March 2009 Declaration, page 3, paragraph 6). Dr. Sipka specifically stated the results indicate a "surprisingly superior effect of IR-LPS over LPS in protecting against the development of hyper-immune response to an allergen neither taught nor suggested by any of the prior art" cited by the Examiner or known to him (March 2009 Declaration, page 4, paragraph 7).

Neither Khan nor Previte teach or suggest the superior effects of IR-LPS compared to native LPS in protecting against the development of allergic disease. Accordingly, Appellants submit the March 2009 Declaration provided by Dr. Sipka constitutes secondary evidence of nonobviousness rebutting any *prima facie* case of obviousness, since it clearly demonstrates that, specifically with respect to the methods of the instant invention, IR-LPS yields unexpectedly superior results in decreasing allergic response, relative to native LPS.

For the reasons set forth above, Appellants respectfully request that the Board reverse the final rejection of claims 1-3, 5, 10, 17-18, and 22-25 as being obvious under 35 U.S.C. § 103 over Khan in view of Previte.

IX. CONCLUSION

For the reasons set forth in detail above, the methods defined by claims 1-3, 5, 10, 13, 17-18, and 22-25 are nonobvious and patentably distinguishable over the teachings of Cochran or Khan and Previte. Accordingly, the final rejections of claims 1-3, 5, 10, 13, 17-18, and 22-25

under 35 U.S.C. §103 should be reversed. Favorable action by the Board is respectfully requested.

Respectfully submitted,

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CLAIMS APPENDIX

Claims on Appeal:

1. (Previously Presented) A process for decreasing development of allergic asthma, the process comprising exposing a neonatal or immature mammal to irradiation-detoxified lipopolysaccharide (IR-LPS) derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein exposure comprises at least weekly administration during maturation of the mammal via application of the IR-LPS to a living environment of the mammal.

2. (Original) A process according to claim 1, wherein the irradiation-detoxified lipopolysaccharide is detoxified by exposure of the endotoxin to irradiation at a level of from about 25 to about 150 kGy.

3. (Previously Presented) A process according to claim 1, wherein the irradiation changes the structure of the endotoxin while maintaining its Th1 stimulatory effect in the resulting irradiation-detoxified lipopolysaccharide.

5. (Original) A process according to claim 1, wherein an infant mammal is exposed.

10. (Previously Presented) A process according to claim 1, wherein application is achieved by administering an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide.

13. (Previously Presented) A process according to claim 1, wherein the mammal is a human and during maturation is between 1 month and 2 years of age.

17. (Previously Presented) A process according to claim 1, wherein exposure to the irradiation-detoxified lipopolysaccharide is initiated shortly after birth and "during maturation" is throughout the maturing life cycle of the mammal.

18. (Previously Presented) A process according to claim 1, wherein administration is on a daily basis.

22. (Previously Presented) A process for decreasing development of allergic asthma in a mammal maturing in an overly sterile environment by restoring normal immune system development, the process comprising exposing a neonatal or immature mammal to irradiation-detoxified lipopolysaccharide derived from extracted *E. coli* bacteria endotoxin and operable to stimulate the Th 1 arm of the mammal's immune system, wherein exposure occurs via administration of the IR-LPS during maturation of the mammal.

23. (Previously Presented) A process according to claim 22, wherein the exposure is achieved by administering an aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide.

24. (Previously Presented) A process according to claim 23, wherein the mammal is a human infant and exposure comprises at least weekly administration from 1 month to 2 years of age via application of the IR-LPS to a living space of the human infant.

25. (Previously Presented) A process for decreasing development of allergic asthma, the process comprising exposing a neonatal or immature human of up to about 2 years of age to irradiation-detoxified lipopolysaccharide derived from extracted bacterial endotoxin and operable to stimulate the Th 1 arm of the human's immune system while reducing interleukin 1(IL-1) stimulation caused by the native form of the lipopolysaccharide derived from extracted bacterial endotoxin, wherein exposure comprises administration on an at least weekly basis of an

aerosol spray composition comprising the irradiation-detoxified lipopolysaccharide at a concentration of 5-15 μg/ml.

EVIDENCE APPENDIX

1. Declaration by Dr. Sándor Sipka under 37 C.F.R. 1.132, filed Mar. 2, 2009

Docket No: 22740-2

CERTIFICATE OF EFS ELECTRONIC TRANSMISSION

I hereby certify that this paper is being transmitted to the United States Patent Office by the USPTO EFS web electronic filing system on March 2, 2009

/Denise M. Everett/

Denise M. Everett

PATENT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant:	Sándor Sipka et al	Confirmation No.:	8175
Serial No.:	10/651,136	Group Art Unit:	1644
Filed:	August 28, 2003	Examiner:	Rooney, Nora Maureen
For:	**Processes For Inhibiting Development of Allergic Disease**		

DECLARATION UNDER 37 C.F.R. 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Dr. Sándor Sipka declares that:

1. He is a co-inventor of and familiar with the present application Serial No. 10/651,136 filed on August 28, 2003, is familiar with the Official Action dated October 2, 2008, and the references cited therein, specifically, Cochran et al, "Influence of Lipopolysaccharide Exposure on Airway Function and Allergic Responses in Developing Mice," *Pediatric Pulmonology*, 34:267-277 (2002), Previte et al, "Detoxification of *Salmonella typhimurium* Lipopolysaccharide by Ionizing Radiation," *Journal of Bacteriology*, 93(5):1607-1614 (1967), and Khan et al, "Functional and immune response to lipopolysaccharide and allergens in developing mice," *Pediatric Research*, 51:474A, (2002).

2. He holds the position of Chief of the Regional Immunological Laboratory, Third Department of Internal Medicine, University of Debrecen, Research Center for

Molecular Medicine, Medical and Health Science Center, Debrecen, Hungary and is knowledgeable in the art of immunology.

3. The present invention is based on the discovery of a unique immune response elicited by irradiation-detoxified (IR) lipopolysaccharide (LPS) and the use of the IR-LPS in a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to the IR-LPS. To demonstrate the unexpected and surprising results of the present methods, the experiments described herein were conducted under his direction and control to test whether IR-LPS exhibits characteristics different from native LPS with respect to in vivo immunomodulatory allergy-prevention.

4. As part of a pre-treatment regimen, water and equal concentrations of native LPS and IR-LPS were sprayed for 8 weeks into the cages of infant mice (age at onset = 6 weeks, Balb/c mice). At the end of the pretreatment period the animals were sensitized by two intraperitoneal injections of 150 μg ragweed allergen (RWE). On day 11 following the sensitization treatment they were challenged with 100 μg RWE. Three days later the cell counts (macrophages and neutrophils/ml) of bronchial lavage (BAL) were measured as well as the serum concentrations of TNFα, a Th1 type cytokine (determined by ELISA). The concentrations of cytokines IL-4 and IL-5 (a Th2 cytokine) were also measured but concentrations for all but IL-5 in the IR-LPS group were below detection limits.

5. The measured results were as follows:

Table 1. The average number of inflammatory neutrophil granulocytes in the bronchial lavage fluid of ragweed sensitized mice after allergen challenge following daily pre-treated with water (H_2O), LPS or IR-LPS sprays for 8 weeks.

Groups of animals treated with	Total number of cells (macrophages + neutrophils)(cells/ml lavage)	Number of neutrophils (cells/ml lavage)
H_2O spray (controls) (5 ml/day) (n=5)	84750	26273

IR-LPS spray (5 μg/5ml H_2O /day) (n=5)	72000* p= 0.04	15840* p = 0.02
LPS-spray (5 μg/5ml H_2O /day) (n=5)	76500	24480

Table 2. The average concentration of tumor necrosis factor alpha (TNFα) (Th1 type cytokine) and interleukin 5 (IL-5) (Th2 type cytokine) in sera of infant Balb/c female mice after an allergic reaction by ragweed following daily pre-treated with water (H_2O), LPS or IR-LPS sprays for 8 weeks

Groups of animals treated with	TNFα (Th1 type cytokine) pg/ml	IL-5 (Th2 type cytokine) pg/ml
H_2O spray (controls) (5 ml/day) (n=5)	4.60	undetectable
IR-LPS spray (5 μg/5ml H_2O /day) (n=5)	16.31* p = 0.001	2.19
LPS-spray (5 μg/5ml H_2O /day) (n=5)	7.60	undetectable

6. The results set forth in the above Table 1 demonstrate that during the allergic reaction to ragweed, the number of inflammatory neutrophils was significantly decreased in the group of mice treated with IR-LPS (p = 0.02) compared to those treated with either H_2O spray or native LPS. The results set forth in Table 2 demonstrate that during the ragweed-specific allergic reaction the serum level of TNFα (Th1 type cytokine) was increased significantly by 3.56 fold (p = 0.001) compared to the controls (16.31/4.58=3.56). However the effect of native LPS was only 1.66 fold (7.60/4.58=1.66). These results illustrate a striking difference between the in vivo immunomodulatory effects of IR-LPS and native LPS on macrophage and neutrophil numbers. This indicates that the prolonged pre-treatment of the environment of infant mice with IR-LPS acts to prevent the intensity of ragweed specific allergic reaction differentially when compared to native LPS. Furthermore, it is clear that IR-

LPS caused a significant increase in the serum concentration of TNFα compared to LPS. It is his opinion that the surprisingly marked difference between the in vivo effects of IR-LPS and native LPS is due to their differing antigenic character which acts on the immunomodulatory Th1 type cells having antigen-specific T cell receptors. It is further his opinion that irradiation of LPS with 150 kGY 60Co-gamma ray results in production or revelation of a new or formerly hidden antigenic determinant(s) in the components of IR-LPS lending the altered and different antigenic character as clearly demonstrated in this study.

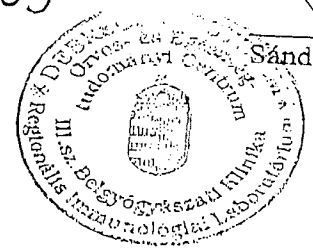
7. The surprisingly superior effect of IR-LPS over native LPS in protecting against the development of a hyper-immune response to an allergen is neither taught nor suggested by any of the prior art cited in the Official Action as noted above or otherwise known to me. Thus, none of this prior art, alone or in combination, suggests any benefit of using IR-LPS, particularly as compared with native LPS, in a method of decreasing development of allergic asthma. Accordingly, none of this prior art, alone or in combination, suggests a method of decreasing development of allergic asthma by exposing a neonatal or immature mammal to IR-LPS.

8. He further declares that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,

23/February/2009
Date

Sándor Sipka



RELATED PROCEEDING APPENDIX